Monitoring of peripheral blood cluster of differentiation 4+ adenosine triphosphate activity and CYP3A5 genotype to determine the pharmacokinetics, clinical effects and complications of tacrolimus in patients with autoimmune diseases

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Abstract. A total of 25 patients with autoimmune diseases receiving tacrolimus were screened using a peripheral blood cluster of differentiation 4+ adenosine triphosphate (ATP) activity assay (IMK assay) between October 2013 and July 2014. The autoimmune diseases of patients were as follows: Rheumatoid arthritis (n=15), lupus nephritis (n=6), ulcerative colitis (n=2) and myasthenia gravis (n=2). Patients were divided into two groups based on CYP3A5 genotype [expression of *1 allele: Expressor (EX; n=6) and non-expressor (NEX; n=19)]. The tacrolimus concentration and concentration/dose ratio was significantly lower in the EX group compared with the NEX group (P=0.0108 and 0.0056, respectively). In addition, all enrolled patients that presented with adverse effects belonged to the NEX group. No significant associations were observed between IMK ATP levels and the concentration or dose of tacrolimus (P=0.1092 and 0.6999, respectively). However, the IMK ATP high-level group exhibited a significantly higher occurrence rate of insufficient effect when compared with the normal and low-level groups (P=0.0014). In conclusion, the clearance of tacrolimus in patients with autoimmune diseases was affected by the CYP3A5 genotype, as previously reported in organ transplant patients. The IMK ATP level may indicate the clinical response irrespective of tacrolimus concentration.

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Introduction

Tacrolimus is used as an immunosuppressive drug in patients following transplant and has a narrow therapeutic window, and is primarily metabolized by cytochrome P450 (CYP) 3A4 and CYP3A5 (1,2). CYP3A5 has been demonstrated to serve a key role in the pharmacokinetics of tacrolimus, specifically in organ transplant patients, and it has been documented that the blood concentration of tacrolimus in patients with a CYP3A5 *1/*1 or *1/*3 genotype (expressors, EX) was lower than that of patients with a *3/*3 genotype (non-expressors, NEX) (3-5). In Japan, tacrolimus has previously been administered to patients with autoimmune diseases, including ulcerative colitis, myasthenia gravis, lupus nephritis and rheumatoid arthritis (6). However, it remains unclear whether the CYP3A5 genotype impacts the pharmacokinetics of tacrolimus in patients with autoimmune diseases in addition to organ transplant recipients.

To determine the optimal dose of tacrolimus in organ transplant patients, physicians typically make dose adjustments based on results obtained from monitoring blood concentration levels of the drug (5). By contrast, the approved dose for patients with autoimmune diseases is fixed, such that 3 mg/day is the dose for myasthenia gravis patients (7). Among these patients with autoimmune diseases, those that present with a CYP3A5 expressor genotype may not exhibit the anticipated effect of the drug, due to a lowered blood concentration of tacrolimus when compared with non-expressors (5). By contrast, the tacrolimus concentration of non-expressors may unpredictably increase and lead to adverse effects, including renal dysfunction and/or infection complications (5).

The ImmuKnow (IMK) assay, which was approved by the Food and Drug Administration (Silver Spring, MD, USA.) in 2002, monitors the function of cluster of differentiation (CD) 4+ T cells by measuring the intracellular concentration of adenosine triphosphate (ATP) (8). The IMK assay has previously been used to identify transplant patients at risk of infection (patients with low IMK ATP levels: <225 ng/ml) or rejection (patients with high IMK ATP

levels: >525 ng/ml) (1,2,9). However, it has been argued that the IMK assay is not a useful indicator of infection or rejection risk (3,4), and for patients with autoimmune diseases, the efficacy of the IMK assay in monitoring immunological aspects remains unclear.

The present study evaluated the association between CYP3A5 genotype and the pharmacokinetics of tacrolimus in patients with autoimmune diseases. Furthermore, the efficacy of the IMK assay in monitoring immunological aspects in patients with autoimmune diseases was investigated.

Materials and methods

Study design. A total of 25 randomly selected autoimmune disease patients who underwent treatment with tacrolimus at the Mie University Hospital (Mie, Japan) between October 2013 and July 2014 were enrolled in the current study. Patients were assessed using IMK and tacrolimus concentration assays following the collection of signed informed consent. Patients were administered tacrolimus with the dose approved by the Japanese Ministry of Health, Labour and Welfare (Tokyo, Japan) as a prescription drug for the treatment of rheumatoid arthritis (3 mg/day), lupus nephritis (3 mg/day), myasthenia gravis (3 mg/day) and ulcerative colitis (0.025 mg/kg twice a day) (6,7,10). The physicians in charge of patients prospectively evaluated the incidence of insufficient effect. Any adverse effects of tacrolimus in each patient were also retrospectively assessed. The Clinical Ethics Review Board of Mie University Hospital approved the present study (No. 2605).

Patients. A total of 25 patients with a median age of 57 (range, 28-88) and 5/20 male: female ratio with autoimmune diseases (rheumatoid arthritis: n=15, lupus nephritis: n=6, myasthenia gravis: n=2 and ulcerative colitis: n=2) who were administered with tacrolimus at the Mie University Hospital between October 2013 and July 2014, were enrolled in the present study. CYP3A5 genotype, peripheral blood CD4$^+$ ATP activity, tacrolimus concentration and clinical effects were evaluated in all patients. Patients who did not take tacrolimus were excluded from this study.

ImmuKnow (IMK) assay. Peripheral blood samples were collected in sodium heparin tubes on admission at the ward or the outpatient clinic, and the intracellular ATP level was measured using an ImmuKnow assay kit (Cylex, Inc., Columbia, MD, USA). Blood samples were processed on the day of sample collection. Briefly, 250 μl anti-coagulated whole blood was diluted with the kit diluent to make a final volume of 1,000 μl. In accordance with the manufacturer's instructions, samples were added to wells of a 96-well plate with phytohemagglutinin (Medical & Biological Laboratories, Co., Ltd.) and incubated for 15-18 h with at 37˚C and 5% CO$_2$ atmosphere. After enrichment for CD4$^+$ T cells by the addition of magnetic particles coated with an anti-human CD4 monoclonal antibody (cat. no. 4402329; dilution as provided in the kit; Dynabeads; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), cells were washed with PBS and bovine serum albumin (concentration as provided in the kit; Cyclex, Inc., Columbia, MO, USA) and lysed to

release intracellular ATP. Released ATP was measured with a luciferin/luciferase assay in a luminometer (Berthold Technologies, LLC, Midway, TN, USA) according to the manufacturer's protocol. The patient's level of immune response was expressed as the amount of ATP (ng/ml).

According to a previous study (3), the subjects were divided into 3 groups, using group boundaries previously established in transplant patients. The present study defined the ATP low-level group as <225 ng/ml, in which patients exhibited an over-immunosuppressed state, the ATP high-level group as >525 ng/ml, in which patients exhibited an under-immunosuppressed state, and the ATP middle-level group as 226-525 ng/ml, in which patients were considered to be within the target immunological response zone.

Administration of tacrolimus. For patients with rheumatoid arthritis, lupus nephritis and myasthenia gravis, the approved maximum dose of tacrolimus administered orally is 3.0 mg/day after food from the day of patient admission. The trough level of tacrolimus in these diseases remains unclear; however, 3.0 mg/day is recommended as this induces minimal renal dysfunction (11). For ulcerative colitis, the recommended dose of tacrolimus is 0.025 mg/kg twice a day orally from the day of admission. The target whole-blood trough level for tacrolimus was 10-15 ng/ml during the first 2 weeks and 5-10 ng/ml after 2 weeks.

Evaluation of tacrolimus blood concentration and concentration/dose (C/D) ratio. The tacrolimus blood concentration was measured using a chemiluminescent immunoassay (ARCHITECT® i2000 tacrolimus Abott 1L77-25; Abbott Laboratories S.A., Shanghai, China) according to the manufacturer's protocol. The daily dose of tacrolimus was adjusted based on tacrolimus blood concentration measurements and its weight-adjusted dose (mg/kg per day) was calculated. The measured blood tacrolimus concentration was then normalized to the corresponding dose per body weight 24 h prior to blood sampling to obtain the concentration/dose (C/D) ratio.

Genotyping of CYP450 3A5. According to a previous study (2), the CYP3A5 A6986G (rs776746) polymorphism was analyzed to detect the *3 allele, as CYP3A5*3 is the major defective allele (2). Furthermore, other functional exonic single-nucleotide polymorphisms (SNPs) are rare in the Japanese population (12). Based on the CYP3A5 genotype, patients were allocated into 2 groups: CYP3A5 *1/*1 or CYP3A5 *1/*3 (EX, n=6) and CYP3A5 *3/*3 (NEX, n=19).

Definitions of insufficient and adverse effects. Insufficient effect was defined as a worsening or lack of improvement in the patient's clinical condition. If worsening of the patients' clinical condition was observed, an increased dosage of tacrolimus was administered or the treatment was changed to other drugs as cyclosporine, or additional immunosuppressants (e.g. Mizoribine, Endoxan) were administered (Table I). Adverse effects were defined as a reduction or loss in the effects of tacrolimus, and when patients required treatment for renal dysfunction, hyperkalemia, tremor, headaches, and/or hyperuricemia.

Table I. Characteristics of patients exhibiting an insufficient effect following TAC therapy.

Patient no.	Genotype	Gender	Age	Adaptation disease	Months after TAC therapy	TAC dose, mg/day	TAC conc, ng/ml	IMK, ng/ml	Clinical condition	Concurrent medication	Additional therapy
1	EX	F	29	LN	33.2	3.0	4.5	535.5	NI	PSL 10.0 mg/day	None
2	EX	F	47	LN	9.7	3.0	1.9	422.5	W	PSL 7.5 mg/day	Change to CYA 4 mg/kg
3	EX	M	64	RA	11.9	2.0	3.1	704.0	W	PSL 5.0 mg/day Iguratimod 25.0 mg/day Etanercept 25.0 mg/week	Increase of TAC 3.0 mg/day
4	NEX	F	66	LN	0.1	2.0	9.9	574.0	W	PSL 30.0 mg/day	Addition of mizoribine 150 mg/day
5	NEX	F	49	LN	0.5	2.0	4.7	1,007.0	NI	PSL 15.0 mg/day	None
6	NEX	F	43	LN	0.6	2.0	2.9	360.5	W	PSL 45.0 mg/day	PE Pulse of CPA 20 mg/kg/3 weeks
7	NEX	F	72	RA	14.4	1.5	7.2	357.5	W	MTX 4.0 mg/week	Increase of MTX 6.0 mg/week

TAC, tacrolimus; EX, expressor; NEX, non-expressor; IMK, ImmuKnow assay; F, female; M, male; LN, lupus nephritis; RA, rheumatoid arthritis; PSL, prednisolone; MTX, methotrexate; CYA, cyclosporine; PE, plasma exchange; CPA, cyclophosphamide; NI, clinical condition did not improve; W, clinical condition worsened.

Table II. Patient characteristics.

Characteristic	CYP3A5 EX (n=6)	CYP3A5 NEX (n=19)	P-value
Sex, male/female	1/5	4/15	1.0000
Age, years	55.5 (29.0-76.0)	57.0 (28.0-88.0)	0.7927
Body weight, kg	50.8 (34.9-99.1)	51.5 (38.2-91.5)	0.6781
Primary disease			
Rheumatoid arthritis	4	11	0.6575
Lupus nephritis	2	4	
Ulcerative colitis	0	2	
Myasthenia gravis	0	2	
Months after starting tacrolimus therapy	46.6 (9.7-88.6)	13.7 (0.1-87.5)	0.0416[a]
Laboratory data			
BUN, mg/dl	14.5 (7.0-20.0)	16.0 (9.0-32.0)	0.2795
CRE, mg/dl	0.7 (0.6-0.8)	0.6 (0.5-1.3)	0.6990
K, mEq/l	3.9 (3.6-4.2)	4.1 (3.2-5.1)	0.0746
AST (U/l)	23.5 (13.0-30.0)	20.0 (6.0-39.0)	0.3396
ALT (U/l)	16.5 (7.0-48.0)	13.0 (4.0-40.0)	0.8160
WBC, mm^{-3}	7,460.0 (3,750.0-9,920.0)	6,150.0 (3,150.0-14,650.0)	0.4367

[a]$P<0.05$ vs. EX group. Values are presented as the median (min-max). CYP3A5, cytochrome P450 3A5; BUN, blood urea nitrogen; CRE, serum creatinine; K, blood potassium; AST, aspartate aminotransferase; ALT, alanine aminotransferase; WBC, white blood cell count; EX, expressor; NEX, non-expressor.

Statistical analysis. All values were expressed as the median (min-max) as appropriate. Fisher's exact tests were used for categorical factors. A Mann-Whitney test was used to compare the results of two groups and a Kruskal-Wallis test was used to compare the results of three groups. The data were analyzed using GraphPad Prism 6.0 software (GraphPad Software, Inc., La Jolla, CA, USA), and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. The characteristics of patients are presented in Table II. There were 6 patients in the EX group and 19 in the NEX group. The two groups exhibited a similar sex ratio, age range, body weight, primary disease and laboratory data during the period that tacrolimus concentration was measured, and no significant differences were observed in the patient variables. However, the duration of tacrolimus therapy (in months after initiation of tacrolimus treatment) was significantly shorter in the NEX group when compared with the EX group (P=0.0416; Table II).

Impact of CYP3A5 genotype on tacrolimus pharmacokinetics and clinical response. The tacrolimus dose did not differ significantly between the EX and NEX groups (P=0.6980; Fig. 1A). However, the EX group exhibited significantly lower tacrolimus concentrations and C/D ratios when compared with the NEX group (P=0.0108; Fig. 1B and P=0.0056; Fig. 1C, respectively). A total of 3 patients presented with insufficient effect (50%) in the EX group and 4 cases (21%) of insufficient

effect were observed in the NEX group, and no significant difference was determined between the two groups (P=0.562; Fig. 2A). Adverse effects developed in 5 cases (26%; renal dysfunction: n=3, hyperkalemia: n=3, hyperuricemia: n=3, with overlap) of the NEX group, and no adverse effects were identified in the EX group during the treatment period. Thus, all incidences of adverse effect were observed in the NEX group (P=0.289; Fig. 2B). Table I presents the characteristics of patients that exhibited insufficient effect following tacrolimus therapy (rheumatoid arthritis in 2 and lupus nephritis in 5 cases). Clinical conditions worsened in 5 cases (rheumatoid arthritis: n=2, lupus nephritis: n=3) and did not improve in 2 cases (lupus nephritis: n=2; Table I).

Association between IMK ATP level and tacrolimus pharmacokinetics, clinical response and CYP3A5 genotype. Patients were categorized into three different categories according to IMK ATP levels (low, normal and high), as previously established in transplant patients (3) (Fig. 3A; P=0.0001). The concentration and dose of tacrolimus did not differ significantly between the low, normal and high ATP groups (Fig. 3B and C; P=0.1092 and 0.6999, respectively). The incidences of insufficient effect were 100% (4/4) in the high ATP group, 20% (3/15) in the normal group and 0% (0/6) in the low group, which were deemed to be significantly different (Fig. 4A; P=0.0014). The incidences of adverse effects due to tacrolimus did not differ significantly among the three groups (Fig. 4B; P=0.9492), and no significant difference was observed in the number of CYP3A5 expressors among the three groups (Fig. 4C; P=0.8105).
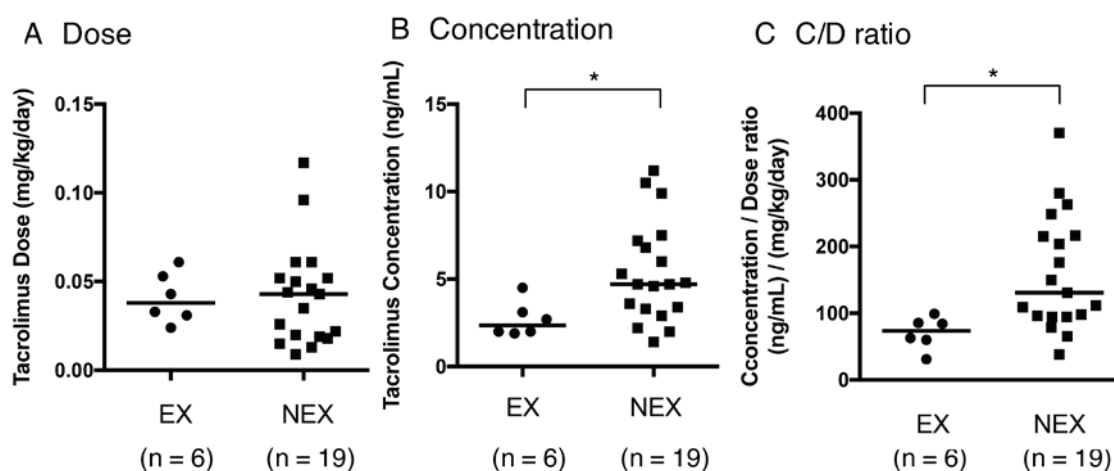
Figure 1. Impact of CYP3A5 genotype on the dose and concentration of tacrolimus. Comparison of (A) tacrolimus dose, (B) tacrolimus concentration and (C) the concentration/dose ratio in the different CYP3A5 genotypes. *P<0.05. EX, expressor; NEX, non-expressor; C/D, concentration/dose.
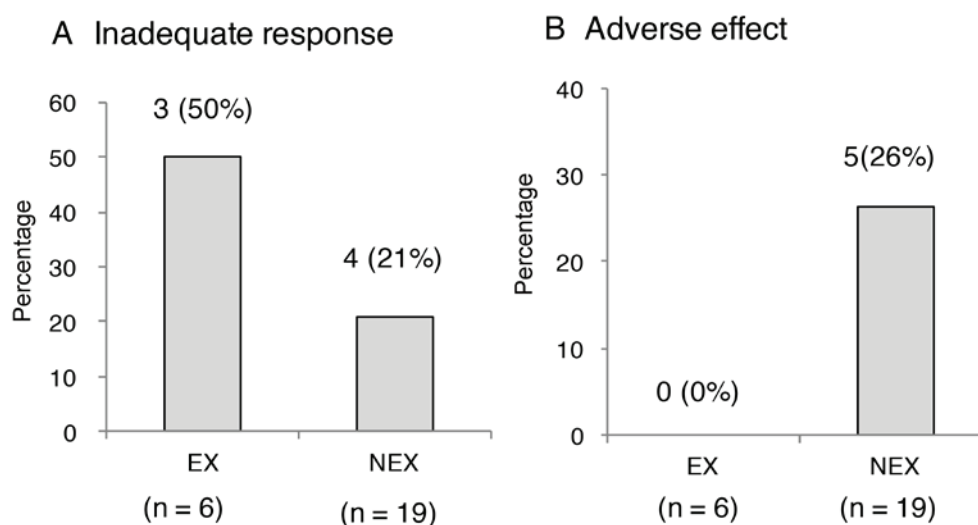

Figure 2. Occurrence rate of insufficient and adverse effects in different CYP3A5 genotypes. (A) Patients that exhibited insufficient effect included 3 cases (50%) in the EX group and 4 cases (21%) in the NEX group. There was no significant difference in the rate of insufficient effect between the two groups (P=0.562). (B) Adverse effects due to tacrolimus developed in 5 patients (26%) of the NEX group. There was no significant difference in the rate of adverse effects between the two groups (P=0.289). EX, expressor; NEX, non-expressor.

Discussion

For patients that have undergone organ transplantation, previous results have suggested that the metabolism of tacrolimus is affected by CYP3A5 genotype (5). However, it is unclear whether CYP3A5 genotype affects patients with autoimmune diseases. Results of the present study suggested that the pharmacokinetics of tacrolimus in patients with autoimmune diseases were influenced by CYP3A5 in a similar way to that in transplant patients. Notably, the range of the IMK assay, as defined in organ transplant patients, may be a useful indicator of clinical response in autoimmune patients.

Tacrolimus is characterized by high inter-individual variation in its pharmacokinetics, which makes it difficult to establish an optimal dose regimen of the drug in transplant patients (13). A factor that contributes to the pharmacokinetic

variability of tacrolimus is considered to be SNPs of CYP3A5 (14). The use of tacrolimus has been approved for the treatment of autoimmune diseases in Japan, and an approved dosage has been fixed, such that 3 mg/day is the dose for myasthenia gravis patients (7). One reason for this is that the approved tacrolimus dosage may be regulated by primarily focusing on adverse events and not clinical effects.

In the present study, the CYP3A5 genotype significantly influenced the clearance of tacrolimus in patients with auto-immune diseases, though the dose did not differ. While not significantly different, the incidences of insufficient effect were notably higher in the EX group when compared with the NEX group, and adverse effects developed only in the NEX group, indicating an inadequate dosage for treatment. There are ethnic differences in the distribution of CYP3A5 SNPs, and the frequency of the expressor genotype has been iden-tified in ~40% of the Japanese population (5,15). Therefore,
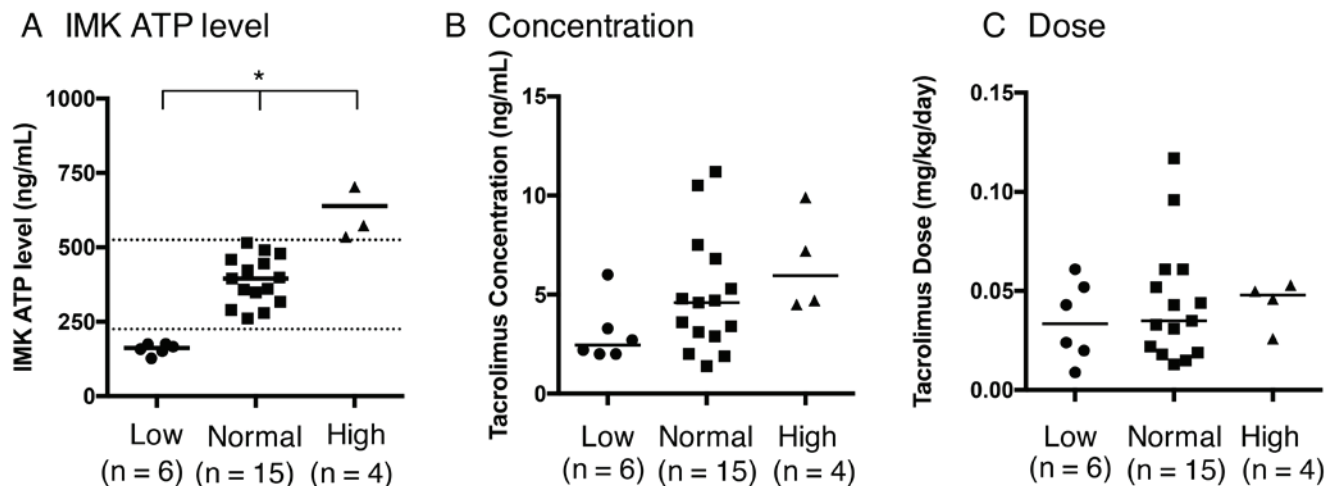
Figure 3. Association between IMK ATP level and tacrolimus pharmacokinetics. Comparison of (A) IMK ATP level with (B) tacrolimus concentration and (C) tacrolimus dose. Patients were divided into three established zones based on IMK ATP level: Low, <225 ng/ml (closed circle), Normal, 225-525 ng/ml (closed square) and High, >525 ng/ml (closed triangle). *P<0.05. IMK, ImmuKnow assay; ATP, adenoside triphosphate.
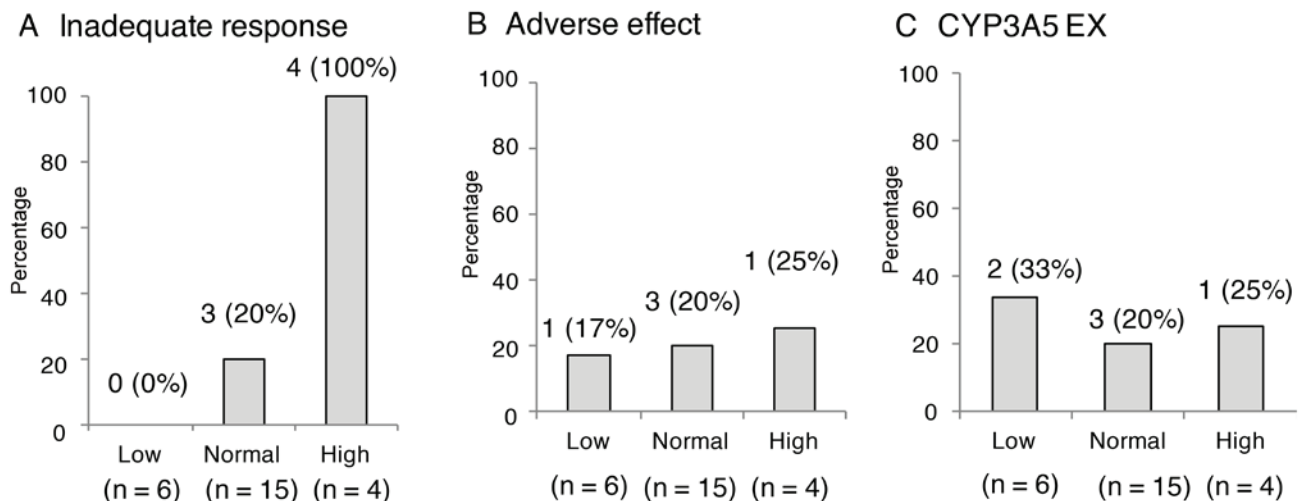

Figure 4. Occurrence rate of insufficient effect, adverse effect and CYP3A5 genotype at different IMK ATP levels. (A) The incidences of insufficient effect were 100% (4/4) in the High ATP group, 20% (3/15) in the Normal group and 0% (0/6) in the Low group, which were deemed to be significantly different (P=0.0014). (B) The incidences of adverse effects due to tacrolimus did not differ significantly among the three groups (P=0.9492). (C) No significant difference was observed in the number of CYP3A5 expressors among the three groups (P=0.8105). Patients were categorized into three established zones based on IMK ATP level: Low, <225 ng/ml, Normal, 225-525 ng/ml and High, >525 ng/ml. CYP3A5, cytochrome P450 3A5; IMK, ImmuKnow assay; ATP, adenosine triphosphate.

CYP3A5 genotype should be identified in patients eligible for tacrolimus treatment, specifically in Japan.

The IMK assay is considered to be a useful tool for monitoring immune activity in transplant recipients (1,3). However, the benefits of the IMK assay in the monitoring of immunological aspects in patients with autoimmune diseases remain unclear. Kowalski *et al* (1) reported that the range of IMK is a useful tool for predicting the immune state of patients following liver transplantation. Therefore, the present subjects were divided into three groups using group boundaries previously established in transplant patients (3). In the current study, the IMK ATP level was not associated with the concentration or dose of tacrolimus, as reported previously (16). By contrast, the IMK ATP level was associated with clinical response. No

cases developed infectious complications due to over-immunosuppression in the present study, indicating that the approved dose for autoimmune patients may be set and fixed based on the safety of drug use. In addition, clinical response was associated with IMK ATP level and not tacrolimus dose. The present results indicate that the IMK ATP level is reflected in the clinical response of patients with autoimmune diseases, which may be a useful indicator for determining the regimen of tacrolimus in autoimmune patients.

However, the present study had a number of limitations that should be considered. Firstly, it was difficult to exclude the potential effects of other unknown cofounders in the current single-institution retrospective study. Secondly, the results remain a matter of speculation due to the small number

of cases assessed for each disease. Thirdly, the current study was not able to evaluate chronological change in each patient. Further multi-study analyses conducted in a prospective randomized controlled fashion and with a greater number of patients are now required.

In conclusion, the clearance of tacrolimus in patients with autoimmune diseases was affected by CYP3A5 genotype, as previously reported for patients who had undergone organ transplantation. The IMK ATP level may be a useful indicator of clinical response, irrespective of tacrolimus concentration, in patients with autoimmune diseases in addition to organ transplant patients.

Acknowledgements

References

1. Kowalski R, Post D, Schneider MC, Britz J, Thomas J, Deierhoi M, Lobashevsky A, Redfield R, Schweitzer E, Heredia A, et al: Immune cell function testing: An adjunct to therapeutic drug monitoring in transplant patient management. Clin Transplant 17: 77-88, 2003.
2. Mizuno S, Hamada T, Nakatani K, Kishiwada M, Usui M, Sakurai H, Tabata M, Sakamoto Y, Nishioka J, Muraki Y, et al: Monitoring peripheral blood CD4+ adenosine triphosphate activity after living donor liver transplantation: Impact of combination assays of immune function and CYP3A5 genotype. J Hepatobiliary Pancreat Sci 18: 226-234, 2011.
3. Kowalski RJ, Post DR, Mannon RB, Sebastian A, Wright HI, Sigle G, Burdick J, Elmagd KA, Zeevi A, Lopez-Cepero M, et al: Assessing relative risks of infection and rejection: A meta-analysis using an immune function assay. Transplantation 82: 663-668, 2006.
4. Israeli M, Yussim A, Mor E, Sredni B and Klein T: Preceeding the rejection: In search for a comprehensive post-transplant immune monitoring platform. Transpl Immunol 18: 7-12, 2007.
5. Muraki Y, Usui M, Isaji S, Mizuno S, Nakatani K, Yamada T, Iwamoto T, Uemoto S, Nobori T and Okuda M: Impact of CYP3A5 genotype of recipients as well as donors on the tacrolimus pharmacokinetics and infectious complications after living-donor liver transplantation for Japanese adult recipients. Ann Transplant 16: 55-62, 2011.
6. Kawakami K, Inoue T, Murano M, Narabayashi K, Nouda S, Ishida K, Abe Y, Nogami K, Hida N, Yamagami H, et al: Effects of oral tacrolimus as a rapid induction therapy in ulcerative colitis. World J Gastroenterol 21: 1880-1886, 2015.
7. Nagaishi A, Yukitake M and Kuroda Y: Long-term treatment of steroid-dependent myasthenia gravis patients with low-dose tacrolimus. Intern Med 47: 731-736, 2008.
8. The US Food and Drug Administration. Class II Special Controls Guidance Document: cyclosporine and tacrolimus assays; guidance for industry and FDA. 2002. http://www.fda. gov/MedicalDevices/DeviceRegulationandGuidance/Guidance Documents/ucm092778.htm.
9. Nobuoka Y, Mizuno S, Nishikawa K, Nakatani K, Muraki Y, Yamada T, Okuda M, Nobori T, Sugimura Y and Isaji S: Immune response following liver transplantation compared to kidney transplantation: Usefulness of monitoring peripheral blood CD4+ adenosine triphosphate activity and cytochrome P450 3A5 genotype assay. Clin Dev Immunol 2013: 936063, 2013.
10. Tanaka H, Tsuruga K, Aizawa-Yashiro T, Watanabe S and Imaizumi T: Treatment of young patients with lupus nephritis using calcineurin inhibitors. World J Nephrol 1: 177-183, 2012.
11. Asamiya Y, Uchida K, Otsubo S, Takei T and Nitta K: Clinical assessment of tacrolimus therapy in lupus nephritis: One-year follow-up study in a single center. Nephron Clin Pract 113: c330-c336, 2009.
12. Fukuen S, Fukuda T, Maune H, Ikenaga Y, Yamamoto I, Inaba T and Azuma J: Novel detection assay by PCR-RFLP and frequency of the CYP3A5 SNPs, CYP3A5*3 and *6, in a Japanese population. Pharmacogenetics 12: 331-334, 2002.
13. Denton MD, Magee CC and Sayegh MH: Immunosuppressive strategies in transplantation. Lancet 353: 1083-1091, 1999.
14. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, et al: Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 27: 383-391, 2001.
15. Uesugi M, Masuda S, Katsura T, Oike F, Takada Y and Inui K: Effect of intestinal CYP3A5 on postoperative tacrolimus trough levels in living-donor liver transplant recipients. Pharmacogenet Genomics 16: 119-127, 2006.
16. Mizuno S, Muraki Y, Nakatani K, Tanemura A, Kuriyama N, Ohsawa I, Azumi Y, Kishiwada M, Usui M, Sakurai H, et al: Immunological aspects in late phase of living donor liver transplant patients: Usefulness of monitoring peripheral blood CD4+ adenosine triphosphate activity. Clin Dev Immunol 2013: 982163, 2013.